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# Using ComBase Predictor and Pathogen Modeling Program as support tools in outbreak investigation: an example from Denmark

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## Abstract

During a 20-case-outbreak of verocytotoxin-producing *Escherichia coli* O26:H11 in 2007 in Denmark, two of the cases were also found to be infected with *Yersinia enterocolitica*. The source was an organic semi-dried fermented sausage and the question was: “Could *Y. enterocolitica* have survived, or even multiplied, during the production of the suspected sausage?” To elucidate this, the ComBase Predictor (CBP) and the Pathogen Modeling Program (PMP) were used as support tools. From information on the company’s website, it was calculated that the water phase salt changed from 4.6% to 8.0% during production and pH changed from 5.5 to 4.7. No nitrite was used. Predictions of growth/reduction of *Y. enterocolitica* and *E. coli* in a matrix covering these pH- and WPS-values were compared at 24°C mimicking fermentation temperature, at 16 and 5°C mimicking drying and storage temperatures, respectively. The results showed that *Y. enterocolitica* would be able to multiply during the first part of the production. Compared to *E. coli* growth of *Y. enterocolitica* was predicted to be slower in the beginning of the fermentation but faster in the end with CBP and faster during the whole fermentation with PMP. CBP predicted that an increase of one log-unit took approx. 50 h at conditions in the beginning of the drying period and approx. 100 h in the middle. During storage growth of *Y. enterocolitica* would only be expected in case of production failures, such as insufficient drying or addition of a too low amount of salt to the batter. A deterministic model was constructed in Microsoft Excel using information on the production of the implicated sausage. This model predicted the level of *Y. enterocolitica* to increase 2.3, 4.2 and 7.8 log-units during fermentation, drying and storage, respectively. At the point of release of the sausage for sale, 1 *Y. enterocolitica* could have increased to 10<sup>6</sup> and the sausage could, therefore, not be ruled out as the source of *Y. enterocolitica* found in two of the outbreak cases.

## Keywords

Outbreak, *Yersinia enterocolitica*, *Escherichia coli*, VTEC, semi-dried fermented sausage.

## Introduction

In February 2007, an outbreak of verocytotoxin-producing *Escherichia coli* O26:H11 was realized in Denmark. The outbreak involved 20 cases primarily children in the age from 2 to 3 years. Using a credit card traceability method, a possible source was traced to be an organic semi-dried fermented sausage. The source was later confirmed when the outbreak strain was recovered from raw material used in the sausage production. Besides *E. coli* O26:H11, *Yersinia enterocolitica* was also found in two of the cases and during the outbreak investigation the following question arose: “Could *Y. enterocolitica* have survived, or even multiplied, during the production of the suspected sausage?” To elucidate this, the ComBase Predictor (CBP) and the Pathogen Modeling Program (PMP) were used as support tools.

## Methods

### *Environmental conditions of sausage*

To determine intrinsic characteristics, such as concentration of water phase NaCl, concentration of NaNO<sub>2</sub> and pH, of the suspected semi-dried sausage the following information was collected from the company’s website and from the label. To produce 100 g

of the organic sausage 127 g meat, 4 g salt (NaCl), 2 g spices and 0.5 g dextrose were used. The ready-to-eat sausages contained 9 g fat, 22 g protein and 2 g carbohydrates per 100 g.

The weight reduction (WR) was calculated on the basis of the recipe of the used batter

$$WR = 100 * ((M + Nb + S + D) - 100) / (M + Nb + S + D) \quad (1)$$

where WR is the weight reduction in %, M is gram of meat, Nb is gram of NaCl, S is gram of spices and D is gram of dextrose used for the production of 100 g of sausage.

The NaCl content, measured in gram, in the ready-to-eat sausage, (Ns) was calculated on the basis of amount of NaCl in the batter (Nb) as well as WR

$$Ns = 100 * Nb / (100 - WR) \quad (2)$$

The water content in the ready-to-eat sausage (Ws) was calculated on the basis of the labelled content of fat, protein and carbohydrates as well as the calculated Ns

$$Ws = 100 - (F + P + C + Ns) \quad (3)$$

where Ws is water content in %, F is gram of fat, P is gram of protein and C is gram of carbohydrate in 100 g of ready-to-eat sausage.

The % water in the batter (Wb) was calculated using formulae (1) and (3)

$$Wb = 100 * Ws / (100 - WR) \quad (4)$$

The water phase NaCl concentrations in batter (WPSb) was calculated on the basis Nb from recipe and formula (4)

$$WPSb = 100 * Nb / (Wb + Nb) \quad (5)$$

The water phase NaCl concentrations in the ready-to-eat sausage (WPSs) was calculated on the basis of formula (2) and (3)

$$WPSs = 100 * Ns / (Ws + Ns) \quad (6)$$

Both WPSb and WPSs are measured in %.

Production of semi-dried sausages can be split into three major steps, fermentation, drying and storage. As there was no information available on the website concerning specific processing temperatures, a standard procedure was adopted. Thus, the widely used fermentation temperature of 24°C was chosen to mimic the fermentation step and 16°C to mimic the drying step. Storage temperature was set to 5°C as indicated on the label.

#### *Predictions from CBP and PMP*

Growth/reduction rates of *Y. enterocolitica* and *E. coli* were predicted using both ComBase Predictor (CBP) as well as Pathogen Modeling Program (PMP). The growth and reduction rates were defined as the time in hours to obtain 1 log-increase and 1 log-reduction of the microorganisms, respectively. In all predictions, lag was excluded. In PMP, the procedure for predicting time to 1 log-increase was to set the difference between 'level of concern' and 'initial level' equal to 1 log CFU. With regard to time to 1 log-reduction in PMP, time to obtain 5 log-reductions was predicted and divided by 5. In CBP, time to 1 log-increase was estimated from the predicted doubling time by division by 0.301 ( $\ln(2) / \ln(10)$ ). No non-thermal inactivation models were available for *Y. enterocolitica* and *E. coli* in CBP.

### Spreadsheet model

A deterministic model was constructed in Microsoft Excel for evaluating the fate of *Y. enterocolitica* and *E. coli* during production conditions similar to the sausage implicated in the outbreak. Interviewing the company collected the information on specific production conditions. Fermentation was carried out at 16°C for 48 h, drying from 16 to 13°C in 8 d, reducing the weight gradually, and chilled storage at 5°C for up to 4 months. Intrinsic characteristics of the ready-to-eat sausage were measured to be pH = 5.1 and  $a_w = 0.96$ . By conversion of the  $a_w$  to WPSs = 6.6% as described by (Resnik & Chirife, 1988) and using the formulae (1) to (6), WPSb was estimated to be 4%. The company informed that pH of the batter usually was around 5.5. Prediction of growth/survival was performed with CBP for *Y. enterocolitica* and PMP for *E. coli*. In all predictions, lag was excluded.

### Results and discussion

Production of semi-dried sausages is a dynamic process where pH, temperature and WPS change with ongoing processing. From the website and label information, WPSb was calculated to be 4.6% and WPSs to be 8%. As there is no drying going on during the fermentation step, WPS will be equal to the WPSb during this processing step. WPS in the product after the drying step will be equal to WPSs, as the sausage is vacuum packaged during the storage period and no further loss of water can occur. There was no information on

Table 1: Time (h) to obtain 1 log-increase (**bold**) or 1 log-reduction (*italic*) at 24°C and 16°C of *Yersinia enterocolitica* and *Escherichia coli*.

pH	NaCl (% w/v)	<i>Yersinia enterocolitica</i>				<i>Escherichia coli</i>			
		24°C		16°C		24°C		16°C	
		CBP	PMP	CBP	PMP	CBP	PMP	CBP	PMP
6.0	4.5	<b>8.7</b>	<b>4.4</b>	<b>19</b>	<b>9.1</b>	<b>7.2</b>	<b>6.0</b>	<b>22</b>	<b>17</b>
	5.0	<b>11</b>	<b>4.6</b>	<b>23</b>	<b>9.6</b>	<b>8.3</b>	<b>6.7</b>	<b>24</b>	<b>18</b>
	6.0	<b>16</b>	<i>o.m.</i>	<b>36</b>	<i>o.m.</i>	<b>11</b>	<i>181</i>	<b>32</b>	<i>455</i>
	6.5	<b>20</b>	<i>o.m.</i>	<b>45</b>	<i>o.m.</i>	<b>13</b>	<i>180</i>	<b>36</b>	<i>454</i>
	7.0	<b>25</b>	<i>o.m.</i>	<b>56</b>	<i>o.m.</i>	<i>o.m.</i>	<i>179</i>	<i>o.m.</i>	<i>453</i>
	8.0	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>176</i>	<i>o.m.</i>	<i>451</i>
5.0	4.5	<b>14</b>	<b>4.6</b>	<b>32</b>	<b>9.3</b>	<b>14</b>	<b>11.7</b>	<b>41</b>	<b>31</b>
	5.0	<b>17</b>	<b>4.7</b>	<b>39</b>	<b>9.6</b>	<b>15</b>	<b>13.0</b>	<b>46</b>	<b>34</b>
	6.0	<b>25</b>	<i>o.m.</i>	<b>59</b>	<i>o.m.</i>	<b>21</b>	<i>94</i>	<b>59</b>	<i>235</i>
	6.5	<b>32</b>	<i>o.m.</i>	<b>75</b>	<i>o.m.</i>	<b>24</b>	<i>94</i>	<b>67</b>	<i>238</i>
	7.0	<b>40</b>	<i>o.m.</i>	<b>94</b>	<i>o.m.</i>	<i>o.m.</i>	<i>95</i>	<i>o.m.</i>	<i>241</i>
	8.0	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>96</i>	<i>o.m.</i>	<i>247</i>
4.5	4.5	<b>21</b>	<b>5.0</b>	<b>48</b>	<b>9.9</b>	<b>23</b>	<b>18.0</b>	<b>70</b>	<b>47</b>
	5.0	<b>25</b>	<b>5.0</b>	<b>59</b>	<b>10</b>	<b>27</b>	<b>20.6</b>	<b>79</b>	<b>53</b>
	6.0	<b>38</b>	<i>o.m.</i>	<b>91</b>	<i>o.m.</i>	<b>35</b>	<i>59</i>	<b>102</b>	<i>147</i>
	6.5	<b>47</b>	<i>o.m.</i>	<b>114</b>	<i>o.m.</i>	<b>41</b>	<i>59</i>	<b>116</b>	<i>150</i>
	7.0	<b>59</b>	<i>o.m.</i>	<b>146</b>	<i>o.m.</i>	<i>o.m.</i>	<i>60</i>	<i>o.m.</i>	<i>153</i>
	8.0	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>o.m.</i>	<i>62</i>	<i>o.m.</i>	<i>159</i>

CBP: ComBase Predictor, PMP: Pathogen Modeling Program, o.m.: outside model.

the fermentation or pH conditions of the sausage on the website, but it was stated that dextrose was added to boost the fermentation. This information was used to estimate the final pH of the sausage to be around 4.7. Initial pH was assumed to be similar to pH of raw meat, *i.e.* around 5.5 – 6.0. As the suspected sausage was organic, no nitrite was added. It was decided to compare growth and survival of *Y. enterocolitica* and *E. coli* in a matrix covering pH-values from 4.5 to 6.0 and WPS from 4.5% to 8.0%. Subsequently, the growth/reduction rates of *Y. enterocolitica* and *E. coli* in this matrix were predicted, at 24°C mimicking fermentation, at 16°C mimicking drying and at 5°C mimicking storage. Both CBP as well as

PMP were used for the predictions (Table 1). The results showed that *Y. enterocolitica* would be able to multiply during the fermentation at 24°C as well as during the first part of the drying at 16°C (Table 1). Compared to *E. coli* growth of *Y. enterocolitica* was predicted to be slower in the beginning of the fermentation (24°C) but faster in the end with CBP and faster during the whole fermentation with PMP (Table 1). During drying and chilled storage only CBP covered WPS concentrations higher than 5% in the case of *Y. enterocolitica*. CBP predicted that an increase of one log-unit took approx. 50 h at conditions in the beginning of the drying period and approx. 100 h in the middle (Table 1). As multiplication of *Y. enterocolitica* is unlikely at WPS above 7% (ICMSF, 1996), no further growth during the rest of drying as well as storage would be expected. However, in case of a drying failure or addition of a too low amount of NaCl in the batter, growth of *Y. enterocolitica* could occur. In a semi-dried sausage with WPS of 7 %, CBP predicted a one log-increase of *Y. enterocolitica* in about 1 month at 5°C (results not shown).

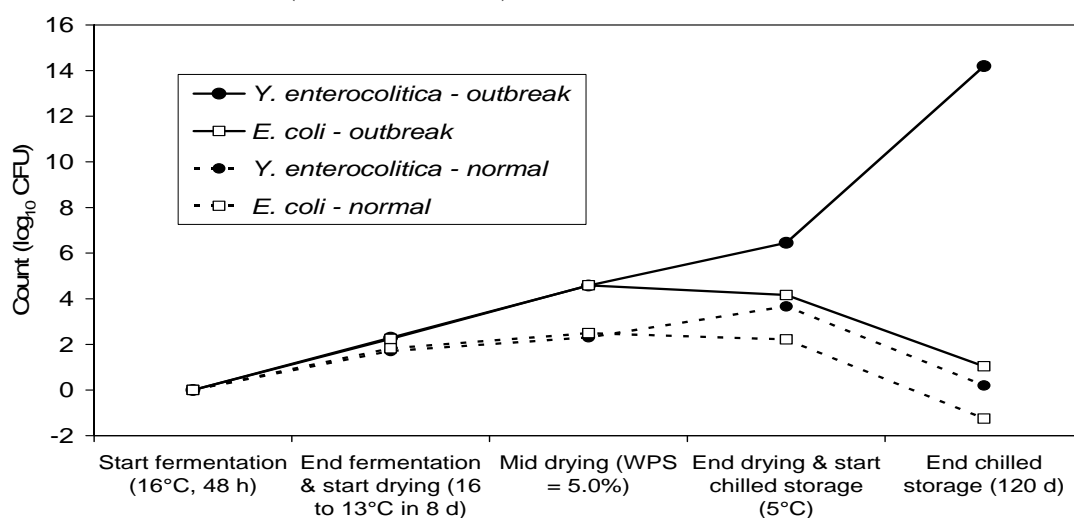


Figure 1: Predicted behaviour of *Yersinia enterocolitica* and *Escherichia coli* under normal production conditions and conditions similar to the semi-dried sausage implicated in the *Escherichia coli* O26:H11 outbreak in Denmark 2007.

When the outbreak source was finally confirmed, a model was constructed in Microsoft Excel that calculated the expected behaviour of *Y. enterocolitica* and *E. coli* during production of the implicated sausage. As shown in Figure 1, the model predicted the level of *Y. enterocolitica* to increase 2.3, 4.2 and 7.8 log-units during fermentation, drying and storage under outbreak production conditions. The corresponding numbers for *E. coli* were 2.2, 1.9 and -3.1 log-units. As can be seen from Figure 1, *E. coli* levels were expected to be highest in the beginning of the shelf-life period. This corresponded well with the fact that most of the cases occurred in the first month after release of the sausage for sale.

## Conclusions

CBP and PMP were successfully used as support tools to evaluate whether two cases of infection with *Y. enterocolitica* could have originated from the same sausage that caused an outbreak of *Escherichia coli* O26:H11. Results showed that this was very likely because, at the point of release of the sausage for sale, production failures had led to an extra 3 log-increase of *Y. enterocolitica* as compared to normal production conditions.

## References

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